Ministry of Health of Ukraine
Poltava State Medical University

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Minutes № from
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METHODOLOGICAL RECOMMENDATIONS FOR CONDUCTING AND PREPARING FOR PRACTICAL CLASSES

Academic discipline	Clinical immunology and allergology
*Module **4***	Clinical immunology and allergology
Content module	Clinical immunology and allergology
*Topic **№1***	Modern views on the structure and function of the immune system
Course	5
Hours	2

Poltava 2023

Methodological recommendations for the practical training for independent work of students in preparation for the practical training and during the class were prepared by:
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Methodological recommendations were re-approved at the meeting of the Department of Internal Medicine of Internal Medicine №3 with Phthisiology_____
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1. Relevance of the topic.

In modern medicine, immunology has taken a significant place as a developing field, but doctors of various specialties rely on it. The relevance of the topic lies in the fact that disorders of the development, differentiation of immunocompetent cells, their functioning, synthesis of their products or regulation of these processes lead to disorders of immunological functions. These disorders may remain asymptomatic or become clinically apparent, and the severity of clinical manifestations ranges from mild to fatal. Such disorders can affect the main cells of the immune system: T- and B-lymphocytes, phagocytes, natural killer cells and their products: complement system proteins, immunoglobulins, cytokines.

A significant number of disorders are associated with congenital or acquired defects in the production of immunocompetent cells or their functions. Other cases of immunodeficiency are associated with malignancy of immunocompetent cells and their uncontrolled proliferation, excessive accumulation of their products. Clinical manifestations of dysregulation of immunological functions can be varied: unregulated activation of the complement system, unregulated production and reception of cytokines.

The immune system consists of organs such as the bone marrow, thymus, spleen, lymph nodes, and lymphoid tissue. There are primary - central (bone marrow and thymus) and secondary - peripheral (spleen, lymph nodes, lymphoid tissue accumulations) organs of the immune system. All of them are interconnected by the circulatory system, lymph flow and a single immunoregulatory system.

General objective: Knowledge of the basics of functioning of innate and acquired immunity, central and peripheral organs of the immune system, age-related aspects of immunology.

Specific objectives:

1. Characteristics of the central and peripheral organs of the immune system.
2. Definition of types of immunity.
3. Factors of innate immunity.
4. Characteristics of antigens.
5. Stages of specific immunity.
6. Structure and function of immunoglobulins.

7. Features of the major histocompatibility complex.

Beginner level of knowledge and skills:

1. Features of the functioning of the central and peripheral organs of the immune system (bone marrow, thymus, lymph nodes, spleen).
2. Factors of innate immunity: cellular (monocyte-macrophage system, killer and granulocyte cells), humoral (complement system, cytokines, etc.).
3. Populations (T- and B-lymphocytes) and subpopulations (T-helper cells of type 1 and 2, T-regulatory cells, T-CTL) of lymphocytes.
4. The concept of innate and acquired immunity.

Theoretical questions for practical training:

1. General concept of the terms clinical immunology and immunity.
2. The main functions of the immune system.
3. Detailed study of the organs of the immune system, their structure, functions in the regulation of immune defence.
4. The concept of immunocompetent cells, variety, functions in the regulation of immune defence.
5. Cytokines: classification, representatives, their structure, functions in the regulation of the immune response.
6. The concept of immunoglobulins, their biological properties.
7. Basic mechanisms of immune response regulation.
8. Stages of immune response formation.

Approximate basis of action

Clinical immunology is a clinical and laboratory discipline that studies the examination, diagnosis and treatment of patients with pathological processes that develop as a result of immune mechanisms disorders, as well as those cases where immunological manipulations are an important part of therapy and/or prevention (WHO, MCIO, IAACI Expert Communiqué, 1993).

Immunity is an evolutionarily determined set of reactions of interaction between the immune system and biologically active agents (antigens) aimed at maintaining the phenotypic constancy of the internal environment (homeostasis) of the organism.

STRUCTURE AND PRINCIPLES OF THE IMMUNE SYSTEM FUNCTIONING

The main functions of the immune system are to control the antigenic state of the body's internal environment, protect the body from pathogenic microorganisms and provide anti-tumour surveillance. These functions involve both nonspecific defence mechanisms and a specific immune response to specific infectious or tumour antigens. The specific immune response enhances the mechanisms of non-specific defence and makes them more targeted.

Organs of the immune system

The central organs of the immune system - the bone marrow and thymus - perform the most important functions, ensuring the self-renewal of the immune system. These organs are responsible for the proliferation of progenitor cells, their differentiation and maturation, up to the release into circulation and settlement of the peripheral organs of the immune system with mature immunocompetent cells.

Bone marrow. All blood cells, including immunocompetent cells, originate from a polypotent stem cell, which gives rise to various hematopoietic lineages, including myelo-monocytic and lymphocytic. The direction of differentiation of early precursors depends on the influence of their microenvironment and the influence of bone marrow stromal cells. The effect of certain cytokines on progenitor cells in vitro is manifested by stimulating the growth of individual colonies consisting of leukocytes of a certain type. Hence their name - colony-stimulating factors GM-CSF, G-CSF, M-CSF. Granulocyte-monocyte factor stimulates the proliferation of early common myelo-monocytopoietic progenitor cells. Granulocyte and monocyte factors stimulate the progenitor cells of each of the sprouts. Even more versatile is the so-called multi-CSF (interleukin-3), which stimulates all hematopoietic sprouts. The producers of these growth factors and other cytokines are bone marrow stromal cells, macrophages and activated lymphocytes. Interleukin-1 and interleukin-6 are synergists of colony-stimulating factors in stimulating proliferation of progenitor cells or inducing the production of growth factors.

The thymus (thymus gland) is the only organ of the immune system that undergoes rapid age-related involution. During the first 50 years of life, 3% of true thymic tissue is lost annually, which is gradually replaced by adipose and connective tissue. The production of T-lymphocytes decreases accordingly. The highest production of T-lymphocytes lasts up to two years of life, and then rapidly decreases. However, it should be noted that the number of T-lymphocytes in the circulation

remains at the achieved level. The fact is that a significant part of the T-cell population consists of cells that live for a long time and do not require constant renewal. Therefore, the number of T cells can be maintained in an adult body even in the absence of the thymus. Moreover, mature T cells undergo so-called "clonal expansion", i.e. selective proliferation in response to an encounter with their antigen, which increases their number.

Peripheral organs of the immune system. The peripheral organs of the immune system - lymph nodes, spleen and lymphoid tissue associated with mucous membranes - are the site of antigen encounter with immunocompetent cells, the site of antigen recognition and development of a specific immune response, the site of interaction of immunocompetent cells, their proliferation (clonal expansion), antigen-dependent differentiation and the site of accumulation of immune response products.

Lymph nodes function as a kind of lymph filters, retaining microorganisms and other particles that have entered the lymph. At the same time, lymph nodes are the site of interaction between immunocompetent cells during a specific immune response, the site of antibody-immunoglobulin synthesis, and the site where cell-mediated immunity events take place.

The lymph node tissue consists of an outer cortical layer, in which clusters of cells form follicles, partly with germinal centres, and an inner medullary layer with a lower content of lymphocytes in combination with macrophages, which are concentrated along the lymphatic and vascular sinuses.

Spleen. The spleen, like the lymph nodes, has T-dependent and B-dependent zones. The spleen is the site of antigen recognition, antigen-dependent proliferation and differentiation of T- and B-lymphocytes, their activation, as well as the production and secretion of specific antibodies, immunoglobulins. The main difference between the spleen and lymph nodes is that the spleen is the site of a specific immune response to antigens circulating in the blood, while lymph nodes are the site of a specific immune response to antigens entering the lymph.

Lymphoid tissue associated with mucous membranes. Accumulations of lymphocytes, macrophages, and other helper cells have been found in many organs and tissues, especially in mucous membranes. Immediately under the mucous epithelium, in close association with epithelial cells, there are lymphocytes of Peyer's plaques of the small intestine, lymphoid follicles of the appendix, tonsils of the pharynx, lymphoid follicles of the submucosal layer of the upper respiratory tract and bronchi, and the genitourinary tract.

Immunocompetent cells

Immunocompetent cells are in a state of recirculation, i.e., they are constantly exchanging cells between blood, lymph and lymphoid organs. This is necessary for the implementation of a specific immune response, as the immune system must be ready to respond to any of the many foreign antigens that enter any part of the body. Since each individual antigen is recognised by only a very small proportion of the lymphocyte population, only constant recirculation can create the conditions for each antigen to meet the 10 lymphocytes carrying antigen-searching receptors specific to it.

In the process of differentiation, various macromolecules appear on the membranes of immune system cells - markers corresponding to a certain stage of development of cell populations.

They are called CD antigens (clusters of differentiation).

- CD3 - is carried by all mature T-lymphocytes, and immature T-lymphocytes in the cytoplasm, provides signal transmission from the T-cell antigen-specific receptor (TCR) to the cytoplasm.

- CD4 is a marker of T helper cells, a transmembrane glycoprotein, one of the receptors of the human immunodeficiency virus (HIV), found on some monocytes and glia cells, and is involved in the recognition of antigens associated with MHC class II molecules.

- CD8 is a marker of T-suppressors and cytotoxic lymphocytes, found on some NK cells, and is involved in the recognition of antigens involving MHC class I molecules.

- CD16 is a marker for natural killer cells (NK cells), monocytes, and the Fc receptor for IgG.

- CD19 is present on pre-B lymphocytes and B lymphocytes, it is part of their receptor complex and is involved in their activation (transduction signal associated with CD21).

- CD22 - is present on mature B-lymphocytes, an adhesion molecule that enhances anti-Lg induced B-cell activation.

- CD25 is present on activated T- and B-lymphocytes and macrophages, participates in the formation of the interleukin-2 receptor, and is released from activated lymphocytes.

Lymphocytes are the only cells in the body that can specifically recognise and distinguish between different antigens and respond with activation upon contact with a particular antigen. With a very similar morphology, small lymphocytes are divided into two populations that have different functions and produce different proteins.

B lymphocytes. In humans, B lymphocytes mature in the bone marrow. B-lymphocytes recognise antigens by specific immunoglobulin receptors, which are expressed on their membranes as they mature. The interaction of an antigen with such receptors is a signal of B-lymphocyte activation and their antigen-dependent differentiation into plasma cells that actively produce and secrete antibodies specific for a given antigen - immunoglobulins.

T-lymphocytes get their name from their differentiation in the thymus. Mature T-lymphocytes (CD2, CD3), unlike immature ones (thymocytes - CD2), are able to respond to T-cell mitogens by proliferating. According to their functions, T-lymphocytes are divided into effector (CD8 cytotoxic lymphocytes - CTL) and regulatory (CD4+ T-helper cells - Th) subpopulations.

T-helper cells stimulate the proliferation and differentiation of cytotoxic lymphocytes, B cells and the formation of antibodies. In other words, T-helper cells have a helper function (stimulate B-lymphocytes to produce immunoglobulins) and an inducer function (stimulate the proliferation and differentiation of cytotoxic lymphocytes that respond to soluble antigens by proliferation and production of lymphokines).

Type 1 T-helper cells (Th1) express the differentiation antigens CD3, CD4, and CD45Ra. They are activators of cellular immunity, natural killer cells and monocytes. Producing interleukins-2, 3, 12, IFN-γ and TNF-α, they cause activation of cytotoxic T-lymphocytes and natural killer cells, proliferation of T- and B-lymphocytes, production of cytokines and synthesis of IgM and IgG2. Thl provide immunity against viruses, intracellular bacteria and oncogenic cells. Th1 activity suppresses interleukin-10.

Type 2 T-helper cells (Th2) have differentiation antigens CD3, CD4, CD29 and are responsible for cooperation with B cells. They activate the humoral immune response and allergic inflammation. By stimulating the production of IgG4 and IgA immunoglobulins by plasma cells, Th2 provide immunity against common (extracellular) bacteria and their toxins.

Natural killer cells (NK cells) are a subpopulation of lymphocytes that are derived from bone marrow progenitors. Their morphological features - large size

and the presence of granules in the cytoplasm - are the basis for their second name, large granular lymphocytes (LGL). Their main functional characteristic is the ability to kill some tumour cells. 14 NKs develop independently of T- and B-lymphocytes and do not carry the surface markers characteristic of T- and B-lymphocytes.

Mononuclear phagocytes. The second major population of cells in the immune system is the mononuclear phagocyte system, which includes bone marrow-derived precursors originating from a single stem cell, the monoblast and promonocyte, the circulating blood monocyte, and mature tissue macrophages. Mononuclear phagocytes provide a largely nonspecific defence of the body through their phagocytic function. Molecules secreted by macrophages perform effector and regulatory functions. In the formation of a specific *immune response, macrophages perform the function of antigen presentation.*

Dendritic cells and Langerhans cells are of bone marrow origin. There are follicular and interdigitated dendritic cells. The former are found in the B-zones of lymph nodes and spleen, they have a receptor for the Fc fragment of immunoglobulins on their surface, but lack MHC class II antigens, and present antigen to B lymphocytes. Interdigitated dendritic cells are found in the T-cell areas of lymph nodes and spleen, have MHC class II antigens on their surface but do not contain receptors for the Fc fragment, and participate in antigen presentation to T lymphocytes.

Granulocytes. Other blood leukocytes can also participate in the effector phase of a specific immune response: granulocytes or polymorphonuclear leukocytes. These cells form the first line of nonspecific antimicrobial defence. They are the first to be mobilised to the site of inflammation or infection, and the elimination of pathogens depends on their phagocytic activity.

Cytokines

Cytokines are products of immunocompetent cells, and at the same time, immunocompetent cells serve as targets of cytokine action. The main mechanisms of action of cytokines can be divided into: growth factors that control the production of immunocompetent cells; pro-inflammatory cytokines that ensure the mobilisation and activation of cells involved in inflammation; anti-inflammatory cytokines with alternative modes of action that limit the development of inflammation; cytokines that regulate the cellular and humoral immune response; cytokines with their own effector functions (antiviral, cytotoxic).

Proinflammatory cytokines.

Interleukin 1 (IL-1). Produced by macrophage cells. IL-1 helps helper T cells to start producing IL-2.

The name interleukin 1 (IL-1) combines two polypeptides: IL-1α and IL-1β, which have a wide range of pro-inflammatory, metabolic, physiological, haematopoietic and immunological activities.

Interleukin 2 (IL-2) is known as a lymphocyte growth factor, i.e. a protein that promotes lymphocyte proliferation. It is produced by Th1.

Interleukin 3 (IL-3) is produced by activated T cells and has the ability to enhance the proliferation of all haematopoietic cells.

Interleukin 5 (IL-5) is an eosinophilic factor. It promotes the activation of eosinophils and prolongs their persistence in foci of eosinophilic inflammation.

Interleukin 6 (IL-6) is a multifunctional cytokine produced by both lymphoid and non-lymphoid cells that regulates the immune response, acute-phase inflammatory response and haemopoiesis. One of the main functions of IL-6 is to regulate the maturation of antibody-producing cells from B lymphocytes and the production of immunoglobulins. IL-6 is also involved in the activation of T lymphocytes.

Interleukin 8 (IL-8) is produced by monocytes, macrophages and other cells: neutrophils, T-lymphocytes, natural killer cells, endothelial cells, fibroblasts, chondrocytes, keratinocytes.

Interleukin 10 (IL-10) is a suppressor interleukin produced in the same way as IL-4 and GL-5 by Th2. It is a cytokine that suppresses Th1 functioning.

Interleukin 12 (IL-12) is produced by monocytes, macrophages, as well as dendritic cells, neutrophils, and activated lymphocytes. Cytokine synthesis is induced by microbial components and products. In recent years, IL-12 has been shown to be a key cytokine for enhancing cell-mediated immune responses and initiating effective defences against viruses, bacteria, fungi and protozoa.

Tumour necrosis factor-alpha (TNF-α), also known as cachectin, is a polypeptide cytokine that performs regulatory and effector functions in the immune response and inflammation. The main producers of TNF-α are monocytes and macrophages, but there are other producers: blood lymphocytes, natural killer cells, blood granulocytes, and T-lymphocyte cell lines.

Immunoglobulins

The products of the humoral immune response are specific antibodies - immunoglobulins. These are large, complex glycoprotein molecules consisting of heavy and light polypeptide chains.

Biological properties of immunoglobulins.

The immunoglobulin (antibody) molecule performs two types of functions: antigen binding based on specific recognition of the antigen epitope by the antibody paratope and effector functions. Recognition and binding of antigenic epitopes is a function of variable regions of the immunoglobulin, while effector functions are determined by a constant region. Antigen binding leads to conformational changes in the constant region, which affect the effector functions of antibodies: complement binding, interaction with FCR, expression of alloantigens, etc.

The physical, antigenic and functional differences between the constant regions define 5 main classes of heavy chains - M, G, A, E and D and the corresponding 5 classes of immunoglobulins.

Ig M. In the course of evolution, IgM antibodies were the first to appear. They are also the first to be synthesised in response to primary antigenic stimulation, i.e. IgM are markers of the primary immune response. They are secreted by B lymphocytes on the 4th-5th day after antigen stimulation.

IgG - antibodies of the IgG class, appear in the serum after IgM during the immune response. IgG are synthesised by mature T-lymphocytes as a result of a specific adaptive immune response, appear in the blood 14-16 days after antigenic stimulation and reach a maximum on the 21st-24th day.

IgA are the main antibodies found in secretions, in the lungs, intestines, and urine. They have an additional structure - a secretory component that protects the antibody molecule from being broken down. The main function of IgA is to prevent the penetration of antigens from external surfaces into tissues.

IgE can bind to mast cells via the Fc fragment and stimulate their degranulation.

IgD act on the surface of B cells, performing regulatory functions.

Stages of the immune response:

1. Presentation of the antigen (antigen presentation). If the antigen is enveloped (a microbe or other particle), it is taken up by macrophages and digested in a phagosome. Small peptides are re-expressed on the membrane in complex with HLA-DR class II antigen and presented to T helper cells (signal I). At the same time, the macrophage is activated and releases IL-1 and other cytokines that activate T helper cells (signal II). Macrophages stimulated by bacteria secrete IL-12, which enhances the differentiation of T-helper cells into Th1 cells. If the antigen is presented by B-lymphocytes, Th2 cells appear.

2. Inducible phase. Th1 and/or Th2, having received 2 signals from macrophages, secrete an appropriate set of cytokines that stimulate the proliferation of T-lymphocytes and B-lymphocytes. Moreover, B-lymphocytes with monomeric IgM as a receptor corresponding to this antigen are activated, i.e., selection and selective stimulation of B-lymphocytes occurs.

3. Effector stage. B-lymphocytes turn into plasma cells that synthesise antibodies, the specificity of which increases in the progeny of dividing cells (the phenomenon of increasing B-lymphocyte affinity). At the same time, antigen-specific T-effectors are generated that carry antigen-specific T-cell receptors (TCRs) on their surface. As a result, antibodies and immune T cells (T-killers) are formed in the body under the influence of antigens.

Simultaneously with the development of the immune response, the mechanisms and suppressor cells that inhibit it are stimulated. Therefore, after a certain period of time, the immune response normally subsides. The body retains immunological memory: T and B memory cells. In the case of initial contact of immunocompetent cells with an antigen, a primary immune response develops.

In terms of time, the primary immune response has a stage of development:

Stage I takes 3-4 days, with no antibodies to the corresponding antigen in the serum.

Stage II - 10-14 days after exposure to the antigen, IgM and IgG appear in the blood serum.

Stage III - the level of antibodies remains constant.

Stage IV takes months and is characterised by a gradual decrease in antibody levels.

A secondary immune response develops upon repeated contact with the antigen, with the formation of class G immunoglobulins. Antibodies, mainly IgG, appear faster and in a higher titer than during the primary immune response.

Test tasks to check the initial level of knowledge

1. Natural immunity is represented:

A. Natural barriers.

B. Mucous membranes.

C. Phagocytosis.

D. Inflammation.

E. There is no correct answer.

2. Antibodies are the main element of defence:

A. Against intracellular antigens.

B. Against extracellular microorganisms.

C. Against tumour antigens.

D. Against humoral immunity.

E. Regarding the central mechanisms of immune response regulation.

3. Decrease in which cell line is observed as a result of aging?

A. Basophils.

B. B-lymphocytes.

C. T-lymphocytes.

D. Monocytes.

E. Eosinophils.

4. The main cells of cellular immunity are:

A. B cells.

B. Macrophages.

C. T cells.

D. Necrosis factor.

E. Eosinophils.

5. Which cells do not belong to antigen-presenting cells?

A. Neutrophils.

B. Dendritic cells.

C. Monocytes.

D. Eosinophils.

E. Leukocytes.

6. Macrophage performs all of the following functions except:

A. Phagocytosis of antigen.

B. Synthesises interleukin-2.

C. Expresses molecules of class 2 of the major histocompatibility complex.

D. Presents peptide fragments of antigen to other cells of the immune system.

E. All answers are correct.

7. Which answer is incorrect? The following subpopulations of lymphocytes are distinguished:

A. T-helper cells.

B. B cells.

C. CD-15 T ligand.

D. CD-4 lymphocytes.

E. Eosinophils.

8. Which cells directly produce class A immunoglobulins?

A. Cytotoxic lymphocytes.

B. CD-4 lymphocytes.

C. Plasma cells of peyer's plaques.

D. Macrophages.

E. Eosinophils.

9. The central organs of the human immune system include:

A. Spleen.

B. Thymus gland.

C. Lymph nodes.

D. Bone marrow.

E. Tonsils.

10. Antigen is a substance that has the following properties:

A. Foreignness.

B. Antigenicity.

C. Immunogenicity.

D. Specificity.

E. All of the above.

Correct answers to the questions: 1 - AUSD; 2 - C; 3 - C; 4 - C; 5 - D; 6 - C; 7 - C; 8 - C; 9 - C, D; 10 - F.

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